## MICROSCOPY.[1]

IMPROVEMENTS IN THE PARAFFIN AND CELLOIDIN METHODS.—The great advantage of the paraffin method is that it permits of making ribbons of thin sections with great rapidity. But material that has passed through this method of imbedding invariably loses something in the *clearness* of its finer histological details, as may be seen by comparison with sections made in elder pith or in celloidin. In some cases structural features are obliterated, or obscured to such a degree as to be beyond detection. As Dr. Apáthy has pointed out, this is especially true of connective tissues and intercellular substances. Can the paraffin method ever be improved so far as to be free from this very serious objection? or can we find a substitute that will have the advantages without the disadvantages of paraffin? Celloidin is free from the objection just mentioned, and it has the inestimable advantage of being a perfect safeguard against brittleness and loss or displacement of loose parts. But the celloidin method is complicated, and does not admit of very thin sections or of ribbon-cutting. Dr. Apáthy has shown us how the serial arrangement of section can be accomplished with the celloidin method, but the process recommended is slow and tedious compared with that of ribbon-cutting. Dr. Kultschizky[2] proposes to combine celloidin with paraffin, and thus to secure the advantages of both and neutralize the defects of each. I have not yet tested Kultschizky's method, but it certainly seems to be the most promising thus far described. It is probable, however, that the *definition* of histological elements will suffer no less by this than by the ordinary paraffin method. Professor Ryder has given the method a trial, and recommends it very highly. Ryder's description is very complete, and, as he suggests some improvements, I shall follow his account.[3]

*The Celloidin-Paraffin Method.*—1. The object to be sectioned is placed in strong alcohol (97 per cent.) until dehydrated or until fully saturated.

2. It is then placed in a mixture of equal parts of ether and alcohol until saturated, the time varying with the size of the object.

3. It is then transferred to a solution of celloidin, prepared as usual in equal parts of alcohol and ether, and in which it is allowed to remain for twenty-four hours.

4. The object is then placed in oil of origanum until saturated, which will be in from one to two or three hours according to the size of the object.

[1] Edited by C. O. Whitman, Director of the Lake Laboratory, Milwaukee.

[2] Zeitschr. f. wiss. Mikroskopie, iv., 1, p. 48, 1887.

[3] J. A. Ryder. Celloidin-Paraffin Methods of Embedding. The Microscopical Bulletin and Science News, Dec., 1887, p 43

5. It is then transferred from the preceding to a mixture of equal parts of oil of origanum and paraffin, which is kept on a water bath for an hour or more, at a temperature of 40°C.

6. It is then transferred to a bath of hard paraffin, or such as melts at 55°C., and is kept there until saturation is complete.

I have tried this method with specimens of injected spleen, and find it to work admirably. The sections can be cut with a dry knife. The sections form a ribbon more easily than in the case of ordinary paraffin imbedding.

The sections may be freed from paraffin with *chloroform* before mounting if they are required for histological purposes, as they may be handled with the greatest ease on account of the presence of the celloidin which holds them together. They can then be stained in hæmatoxylin (Kleinenberg's) or in nigrosin, or double staining effects may be produced by the use of other dyes in combination with hæmatoxylin.

To many persons the oil of origanum has a disagreeable odor, and is almost as inflammable as turpentine; besides, it darkens or oxidizes in a short time, and has, I think, a tendency to shrink the object slightly, even after treatment with celloidin, and also to darken it.

These disadvantages I have lately avoided by substituting chloroform for the oil of origanum, used by Dr. Kultschizky. I proceed in the same manner as he recommends with the imbedding process as regards the first, second, and third steps. The fourth step is to place the object soaked with celloidin in the usual way in chloroform until saturated, instead of in oil of origanum. It is then transferred to a mixture of paraffin and chloroform, equal parts, kept at a temperature of 40°C., and finally, until complete saturation is effected, in molten hard paraffin melting at 55°C.

To clean the sections for mounting, they may be mounted directly from the chloroform, if the operator is quick enough and does not let the chloroform evaporate from the section before it is covered with balsam. A preferable clearing agent, first proposed by Wiegert, I have found to be a mixture of equal parts of *xylol* and pure white *carbolic acid*, which has been allowed to deliquesce or rendered liquid by heat. This may be applied to the section on the slide with a clean camel's-hair pencil, and will clean the section instantly without in the least attacking the celloidin.

*Serial Sections with Celloidin.*[1]—The celloidin block, with the object imbedded, is cut as regularly as possible, and fastened to a cork. In sectioning, the knife should be placed nearly parallel with its direction of motion, and after every five to ten sections wet with 95 per cent. alcohol. The sections are raised from the knife with a small brush, and placed on the surface of bergamot oil (in a small glass dish over a white ground). If the oil is good the sections will at once unroll and become transparent.

[1] J. Apáthy. Methode zur Verfertigung längerer Schnittserien mit Celloidin. Mitth. a. d. zool. Station z. Neapel., vii., 4, p. 742, 1887.

Bergamot oil is in every respect the best to use in celloidin technique. Origanum oil may be used, but its action is violent and often causes the colors to fade. Good bergamot oil is clear grass-green, with at most a slight yellow tinge (yellow oil is always bad), does not smell of turpentine, and mixes with 90 per cent. alcohol without turbidity or formation of water drops on the surface. The little cloudiness produced by breathing on the latter, should at once disappear. Aniline colors ought not to fade perceptibly in bergamot oil even after forty-eight hours; while celloidin ought not to be softened by it in the least; on the contrary, sections that have been softened by strong alcohol should acquire greater firmness in bergamot oil.

Tracing paper must first be cut into strips about as broad as the object-carrier, and at least three times as long as the cover-glass. The shape of the latter is marked on one end of the strips. The paper must be perfectly smooth, well oiled and transparent, and unite some stiffness with flexibility. The strip should be held by its free third, horizontally, in the oil, supported from beneath by the middle and third fingers, and held from above by the thumb and first finger, so that a slight longitudinal and upwardly directed concavity can be given to it. Thus the immersed end of the paper, on which the sections are to be arranged, can easily bear a slight weight without bending. Now, while the left hand holds the strip of paper over the surface of the oil, the right draws the sledge of the microtome with the little finger, and also turns the micrometer screw. Between the middle and third fingers of the same hand, a fine elastic brush is held, supported by the ball of the thumb, and between the first and middle fingers and the thumb a very sharp but strong dissecting needle. The section is removed from the knife, where it lies in plenty of 95 per cent. alcohol, with the brush, and put on the oil; here it is followed with the strip of paper held beneath and guided near the position where it should lie; then drawn with the needle out of the oil on to the paper. The sections are arranged in cross rows, which are held from 2 to 3 mm. out of the oil to prevent them from swimming away. The rest of the paper remains in the oil and is only withdrawn as it is covered with sections. When the desired number of sections has been brought into order on the paper, the oil is drained off, and the paper is then turned so that the sections face downwards. In this position the strip is allowed to fall slowly on the object-glass. Then it is flattened out with a dissecting needle and dried with blotting-paper. Now the tracing-paper, through which the whole series can plainly be seen, must be carefully removed, leaving the sections on the object-glass. If any sections should remain on the paper, the latter, after the sections in question have been moistened with oil, is replaced in its former position on the object-glass, pressed a little, and then removed, or if the sections are quite dry they may be taken with pincers and transferred to the object-glass.

As soon as all the sections are in order on the object-glass, the smooth surface of the blotting-paper is laid on it and stroked lightly several times with the finger to remove all the superfluous oil.

The Canada balsam and cover-glass maynow be added without danger of displacing the sections. Entire removal of the oil insures the preservation of the most fugitive colors. The sections should not be placed near the edge of the cover-glass, as every discoloration begins at the edge.

In the foregoing manner, over 100 sections were placed in a complete series under one cover-glass, in as short a time as by the paraffin method.

As celloidin, like paraffin, does not readily penetrate chitinous envelopes, cuticula and cocoons, care should be taken :—

1. To use at first very thin solutions, which should be gradually brought to the concentration which the imbedding mass is to have

2. To imbed twice, the first time merely to cut the object in pieces, or open a cocoon, or cut etc., with the microtome.

---

## SCIENTIFIC NEWS.

—The American Association for the Advancement of Science will hold its thirty-seventh meeting at Cleveland, commencing August 21st, under the Presidency of the Hon. J. W. Powell, of Washington, D. C.

—The International Geological Congress will hold its fourth meeting in London, commencing September 14th. The honorary president is Professor T. H. Huxley, who is also president of the organizing committee. The Acting president is Professor J. Prestwitch.

—The British Association for the Advancement of Science holds its annual meeting at Bath this year, commencing September 5th. The President is Sir Frederick J. Bramwell.

—The Annual Excursion of the Geological Society of France will be this year in the neighborhoods of Commentry, Chateauneufe St. Germain des Fossés, Moulin and St. Armand. A great range of geological formations will be visited.

—Professor Charles Linden, of the Buffalo High School, died in Buffalo, N. Y., February 3d, 1888, aged fifty-six years. He was born in Breslau, Germany, and came to this country at an early age. His studies were in the line of ornithology, and he made collecting expeditions to Florida, Brazil, the West Indies, and Labrador.